REMARKS

Claims 1-13 and 26-28 were pending in the present application. Claims 14-25 and 29-33 had been previously canceled from the present application and claims 5 and 10-13 have been presently canceled. Accordingly, claims 1-4, 6-9 and 26-28 will be pending upon entry of the instant amendment. Any cancellation of the claims should in no way be construed as an acquiescence to any of the Examiner's rejections and was done solely to expedite prosecution of the application. No new matter has been added, and Applicants submit that all of the claims are now in condition for allowance.

Objections to the Disclosure

The Examiner objected to the specification because the total number of words within the abstract exceeded 150 words.

The abstract has been amended to address the objection of the Examiner. It is believed the amendments contained herein render the present objection moot. Reconsideration and withdrawal of the objection is respectfully requested.

The Rejection of Claims 1-4 under 35 U.S.C. §101, Should Be Withdrawn

Claims 1-4 were rejected under 35 U.S.C §101 because the claimed invention is directed to non-statutory subject matter. Specifically, the Examiner asserts that "Claims 1-4 are directed to a transgenic animal, the scope of which encompasses a human being." The Examiner suggests adding the limitation "non-human" to these claims to remedy the situation. Applicants have amended claims 1 and 26 as suggested by the Examiner, thereby rendering the rejection moot. Reconsideration and withdrawal of the 35 U.S.C §101 rejection is respectfully requested.

The Rejection of Claims 1-13 and 26-28 under 35 U.S.C. §112, First Paragraph Should Be Withdrawn

Claims 1-13 and 26-28 were rejected under 35 U.S.C §112, first paragraph, because the specification,

> [w]hile being enabling for a homozygous transgenic mouse whose germ cells comprise a mutated rchd534-LacZ gene which lacks the MH2 domain encoding region, wherein the endogenous wild-type rchd534 gene of said mouse has been replaced with said mutated rchd534-LacZ gene which lacks the MH2 domain encoding region,

> and wherein said mouse displays a cardiovascular disease symptom as follows: hyperplasia, thickening of at least one cardiac valve, cardiac outflow tract development defects, cardiovascular calcification, epicardial vascular malformations, endocardial vascular malformation, or defects in the regulation of vascular tone, and methods of making and using the same, does not reasonably provide enablement for all other transgenic animals embraced by the claims.

Applicants respectfully traverse this 35 U.S.C §112, first paragraph rejection for the following reasons. Contrary to the Examiner's assertion, Applicants have provided teachings for every element needed for one of skill in the art to practice the claimed invention. In section 5.4.4.1 of the present application (pages 40-44), for example, Applicants have taught how one of skill in the art can generate transgenic animals, characterize such animals for expression (or lack thereof) of the gene of interest and phenotypic analysis and then breed the transgenic animals. First, Applicants have taught that not only mice can be used for the generation of transgenic animals, but that other animals, such as "rats, rabbits, guinea pigs, pigs, micro-pigs, goats, and non-human primates, may be used to generate cardiovascular disease animal models" (see page 41, lines 14-16). Second, Applicants disclose the various techniques, which were known in the art at the time of filing, to introduce a target gene transgene into animals to produce the founder lines of transgenic animals. The techniques described include, for example, pronuclear microinjection, retrovirus mediated gene transfer into germ lines, gene targeting in embryonic stem cells, electroporation of embryos and sperm-mediated gene transfer. Applicants additionally provide citations of references for each technique taught (see page 41, lines 17-25). Third, once the skilled artisan has produced a transgenic animal, Applicants teach one of skill in the art how one would go about to determine whether the animal expresses the recombinant target gene by using standard assay techniques, such as Southern blot analysis, PCR analysis, Northern blot analysis, *in situ* hybridization analysis or RT-PCR analysis (see page 42, lines 10-18). Applicants then teach that once the animal has been analyzed for the target gene's expression, that the animal should then be "further evaluated to identify the animals which display characteristic cardiovascular disease symptoms" (see page 42, lines 19-24). Finally, once an animal has been selected based on the previously described techniques, Applicants further describe various breeding methods that would enable one of skill in the art to obtain the type of transgenic line desired (see page 43, lines 1-16).

As further evidence that one of skill in the art, at the time of filing of the present invention, could use methods taught in the application to generate non-murine transgenic animals, such as sheep, cows or goats, Applicants submit herewith copies of the following four references: 1) Murray, J.D. *et al.* (1989) *Reprod. Fert. Dev.* 1:147-155; 2) Clements, J.E. *et al.* (1994) *Virology* 200:370-380; 3) Janne, J. *et al.* (1992) *Annals of Medicine* 24:273-280; and 4) Ebert, K.M. *et al.* (1991) *Biotechnology* 9:835-838 (all of which are cited in a Supplemental Information Disclosure Statement filed herewith). The authors of these four references used methods as described in the present specification, such as microinjection, to generate the transgenic animals disclosed in each reference. Murray, J.D. *et al.*, for example, disclose the use of pronuclear microinjection for the successful generation of three transgenic sheep expressing high levels of the MTsGH9 fusion protein. Likewise, Clements, J.E. *et al.* generated transgenic sheep via microinjection that expressed the envelope genes of visna virus under the control of the visna LTR. Additionally, Ebert, K.M. *et al.* successfully generated transgenic goats via microinjection that express the Human Longer Acting Tissue Plasminogen Activator (LAtPA) gene in goat milk. Finally, Janne, J. *et al.* describe in their review article from 1992, that using transgenic animals is a viable method for the production of therapeutic proteins. Specifically, Janne, J. *et al.* describe how transgenic animals, such as goats and cows, have been generated to produce human proteins in their milk as an alternative to using microbial recombinant DNA technology. Therapeutic proteins produced by such transgenic animals have the added advantage of being post-translationally modified.

Therefore, contrary to the Examiner's assertions, Applicants have provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of pending claims 1-4, 6-9 and 26-28. Therefore, Applicants respectfully request reconsideration and withdrawal of the foregoing 35 U.S.C. § 112, first paragraph rejection over claims 1-13 and 26-28.

The Rejection of Claims 1-13 and 26-28 under 35 U.S.C. §112, First Paragraph Should Be Withdrawn

Claims 1-13 and 26-28 were rejected under 35 U.S.C §112, first paragraph, because the specification "has not taught creation of a transgenic knockout non-human animal by methods that do not require embryonic stem cells." Specifically, the Examiner asserts that "It is well known in the knockout art that the production of knockout animals other than mice is

undeveloped." Applicants respectfully traverse the Examiner's assertion for the following reasons.

Contrary to the Examiner's assertions, Applicants have taught several different methods for the generation of transgenic animals. Applicants teach such methods on page 41 of the specification and provide citations of references for each method taught. The exemplified methods include, for example, pronuclear microinjection, retrovirus mediated gene transfer into germ lines, gene targeting in embryonic stem cells, electroporation of embryos and sperm-mediated gene transfer (refer to page 41, lines 17-25). At the time of filing of the present application, methods such as pronuclear microinjection, had been proven to be effective for the generation of non-murine transgenic animals. For example, Murray, J.D. *et al.* (1989) *Reprod. Fert. Dev.* 1:147-155 used this pronuclear microinjection technique to generate three transgenic sheep expressing high levels of the MTsGH9 fusion protein. In fact, Murray *et al.* state on page 153, "Although further work is required to determine if higher DNA concentrations will lead to significantly higher integration frequencies in sheep it is clear that transgenic sheep can be reliably produced at a frequency that allows the use of transgenics for both research and commercial purposes." Likewise, Clements, J.E. *et al.* (1994) *Virology* 200:370-380 generated transgenic sheep via microinjection that expressed the envelope genes of visna virus under the control of the visna LTR and Powell B. C. *et al.* (1994) *Reprod. Fertil. Dev.* 6:615-623 generated transgenic sheep via microinjection to study wool growth. Additionally, Ebert, K.M. *et al.* (1991) *Biotechnology* 9:835-838 disclose the generation of transgenic goats via microinjection that express the Human Longer Acting Tissue Plasminogen Activator (LAtPA) gene in goat milk. In fact, Ebert *et al.* state on page 835 "In this paper, we describe the first successful generation of transgenic goats at frequencies that approach those in the rodent systems. More importantly, a transgenic goat was generated that produced an enzymatically active form of tPA throughout a normal lactation period. These experiments further support the concept of targeting expression of transgenes that encode pharmaceutical proteins to the mammary gland of dairy livestock." The concept of using animals as bioproducers of therapeutic proteins by generating transgenic animals was developed prior to the filing of the instant application. As Janne, J. *et al.* (1992) *Annals of Medicine* 24:273-280 describe in their review article, transgenic animals, such as goats, sheep and cows, had been successfully generated and used to produce therapeutic proteins in their milk as early as 1992. In fact, as described on page 275 of Janne, J. et al., "In September 1991 three reports were published simultaneously, representing a real breakthrough in the field

and demonstrating the feasibility of using farm animals as bioproducers of pharmaceuticals." One of these reports includes the generation of transgenic sheep capable of secreting α1-antitrypsin into their milk. The transgenic sheep were all healthy and one of the transgenic sheep produced high quantities of human α1-antitrypsin into its milk. The human α1-antitrypsin "displayed full biological activity and was glycosylated like its plasma derived counterpart". In fact, this transgenic sheep line was so successful that "[i]t is currently in the process of commercialization".

Therefore, Applicants submit that the state of the art at the time of filing was such that one of skill in the art could successfully generate non-murine transgenic animals using methods that do not require embryonic stem cells, such as pronuclear microinjection, and that Applicants have taught one of skill in the art how to generate such animals using such techniques.

Therefore, although Applicants have exemplified the use of embryonic stem cells for the generation of rchd534 transgenic knockout mice, contrary to the Examiner's assertions and as demonstrated herein, Applicants have taught one of skill in the art how to generate non-murine transgenic animals using methods that do not require embryonic stem cells. Such methods were well known in the art and would not constitute undue experimentation on the part of the skilled artisan. Therefore, Applicants respectfully request reconsideration and withdrawal of the foregoing 35 U.S.C. § 112, first paragraph rejection over claims 1-13 and 26-28.

The Rejection of Claims 1-13 and 26-28 under 35 U.S.C. §112, Second Paragraph Should Be Withdrawn

Claims 1-13 and 26-28 were rejected under 35 U.S.C §112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." Specifically, the Examiner asserts that claims 1, 10 and 26 are "[u]nclear as written because it is not clear that the mutated rchd534 gene is the same as the rchd534-LacZ gene and because it is not clear that the endogenous wild-type rchd534 gene has been replaced." Applicants have amended claims 1 and 26 by specifying that "the mutated rchd534 gene is a rchd534-LacZ gene which lacks the MH2 domain encoding region" and Applicants have canceled claim 10, thereby rendering the rejection moot. Reconsideration and withdrawal of the 35 U.S.C §112, second paragraph rejection over claims 1-13 and 26-28 is respectfully requested.

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Double Patenting

Claims 1-13 and 26-28 were rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-11 of U.S. Patent No. 6,359,194. Specifically, the Examiner asserts that "The transgenic animals as claimed in the instant application embrace the genus of animals while the claims of U.S. 6,359,194 are directed to a species of animals, a mouse. Therefore, the claims directed to the transgenic mouse would anticipate the claims directed to the transgenic animals." Applicants will file a Terminal Disclaimer if claims of the instant application which are deemed to be conflicting with claims 1-11 of U.S. Patent No. 6,359,194 are indicated as being allowable.

Practitioner's Docket No. MPI1995-001CP1CP1CN1M **USSN: 10/067,741**

CONCLUSIONS

In view of the amendments and remarks made herein, Applicants respectfully submit that the objections and rejections presented by the Examiner are now overcome and that this application is in condition for allowance. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned.

This paper is being filed timely. No extensions of time are required. In the event any extensions of time are necessary, the undersigned hereby authorizes the requisite fees to be charged to Deposit Account No. 501668.

Entry of the remarks made herein is respectfully requested.

Respectfully submitted,

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